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Title: Bioscience at Los Alamos National Laboratory

Author(s): McDonald, Rebecca E.

Intended for: Visit to SRI International

Bioscience Division

Bioscience at Los Alamos National Laboratory

José A. Olivares

Bioscience Division Leader

Los Alamos NATIONAL LABORATORY EST.1943

LANL Capabilities Support National Security Missions and National Needs

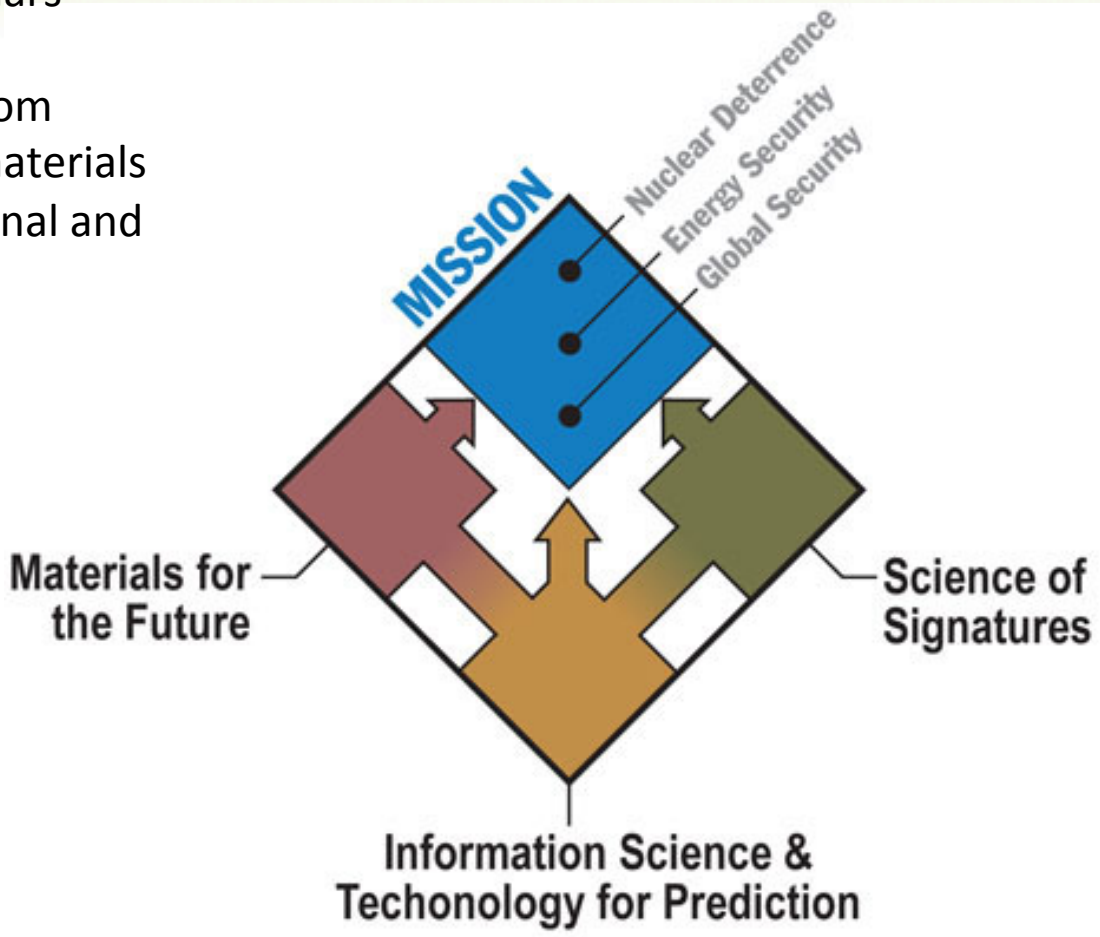
Scientific Credibility | Delivery of Product | Agility and Innovation

LANL is the Oldest, Most Complex, and Second Largest NNSA Site

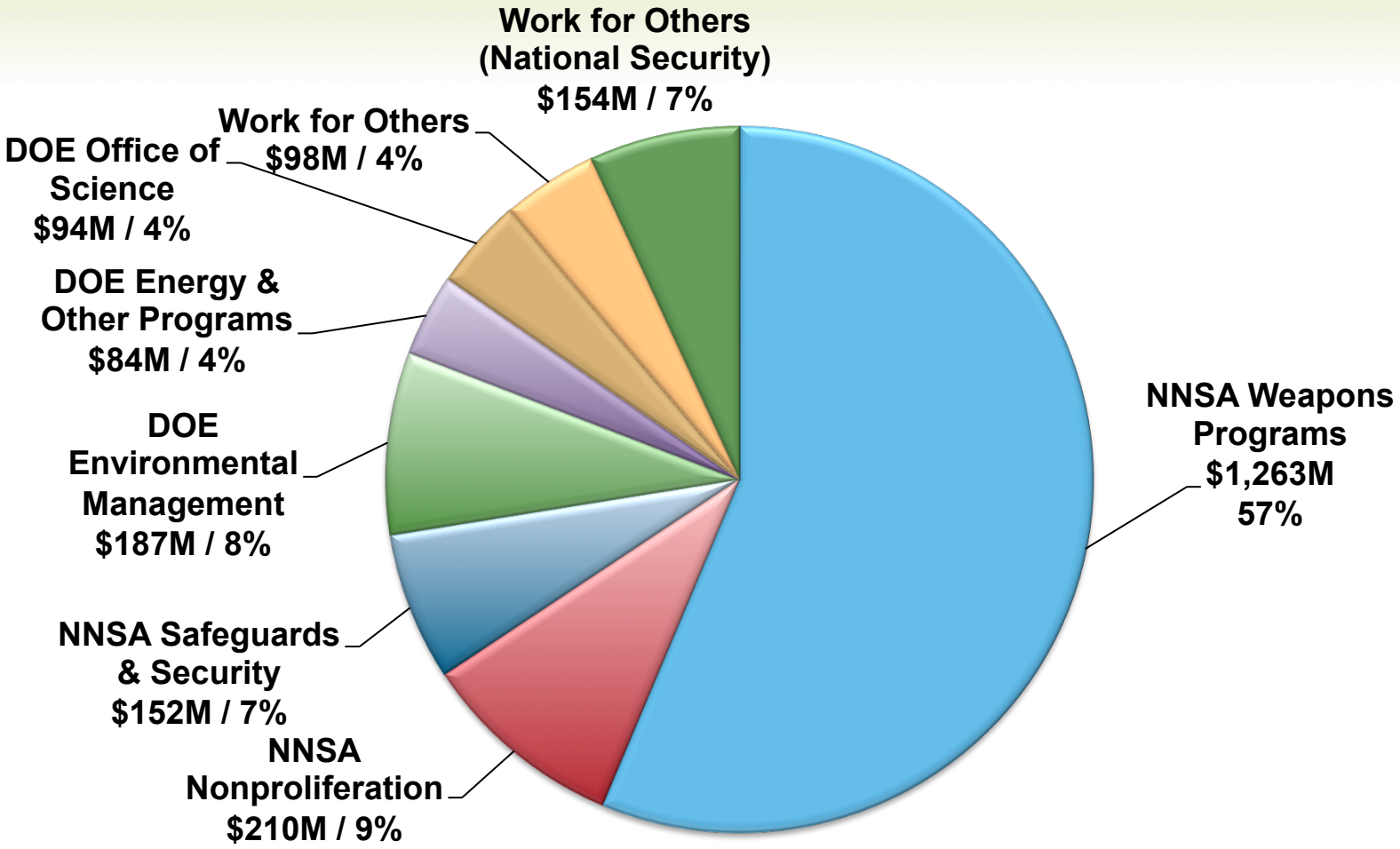
- **~ 40 square miles**
  - 7,500 ft elevation
- **1,280 buildings with 9.0M gross sq. feet**
  - 11 nuclear facilities
  - 40% are more than 40 years old
  - 30% of staff work in poor or failing space
  - $450M of Deferred Maintenance backlog
- **268 miles of roads (100 paved)**
- **Utilities**
  - 26 miles of 115-KV transmission lines
  - 90 miles of gas transmission lines

Science Pillars

- LANL's three science pillars harness capabilities for solutions to threats—from cyber security to new materials development—on national and global scales

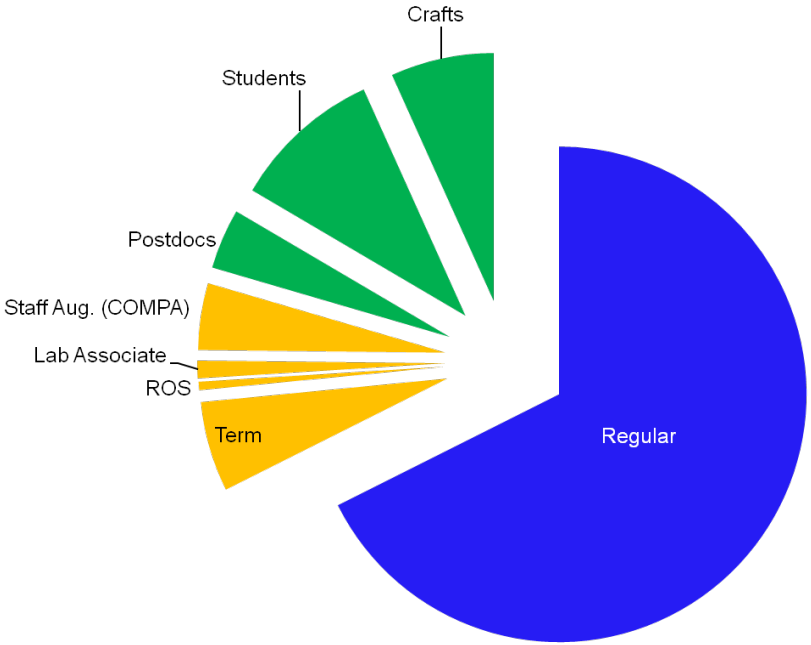


LANL Budget FY12 - $2,242M

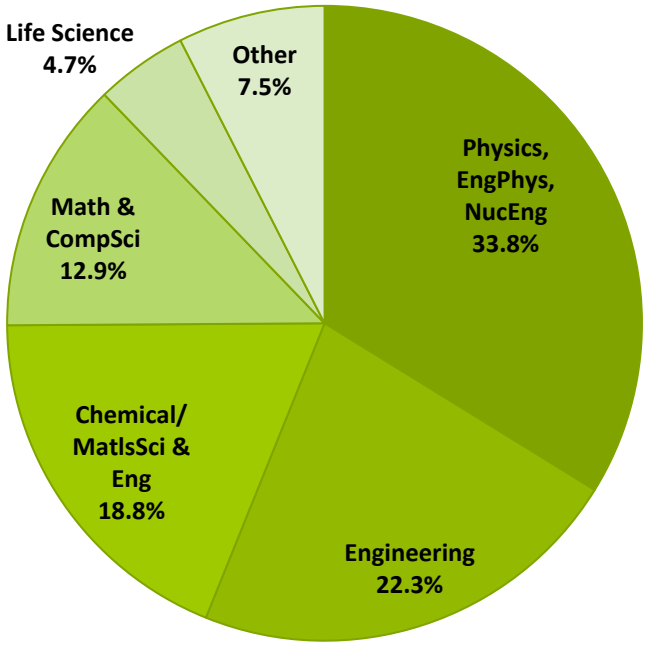


LANL workforce shows depth and breadth

LANL Staffing Levels
11,127 Employees

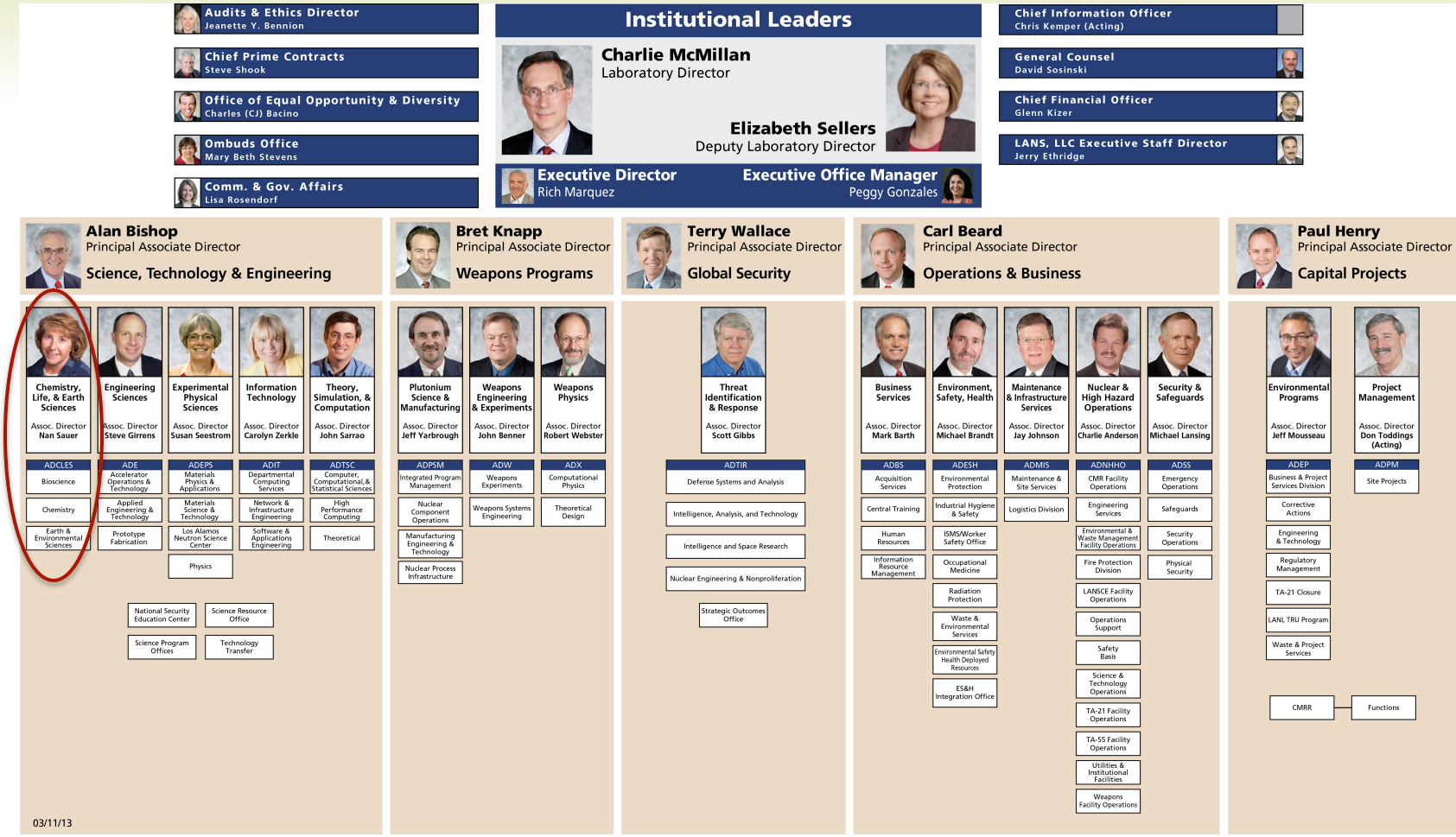


R&D Technical Staff Disciplines



Bioscience Division

Los Alamos National Laboratory



Chemistry, Life and Earth Sciences Directorate

Associate Director for Chemistry, Life, and Earth Sciences
Nan Sauer
Deputy AD, Tammy Taylor (Act.)

Bioscience
José Olivares, DL
Jurgen Schmidt, DDL

- *Bioenergy*
- *Biosecurity*

Chemistry
Carol Burns, DL
T. Mark McCleskey, DDL

- *Actinide Analytical Chemistry*
- *Physical Chemistry and Applied Spectroscopy*
- *Chemical Diagnostics and Engineering*
- *Nuclear and Radiochemistry*
- *Inorganic and Isotope Chemistry*

Earth & Environmental Sciences
James E. Bossert, DL
Andy Wolfsberg, DDL

- *Repository Science and Operations*
- *Earth Systems Observations*
- *Computational Earth Sciences*
- *Geophysics*

LANL Contributions to Bioscience Research

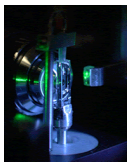
Bioscience Division

Radiation health effects

1945

1953
First liquid scintillators

1967

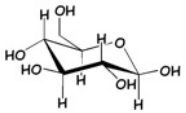


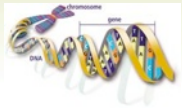
Flow cytometry (Resource: 1984)

Immunological modeling

1970

1974
Stable Isotope Resource





National Laboratory Gene Library Project

1983

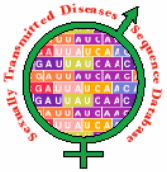
GenBank

1982

Human Genome Project

1988

1980's-1990's



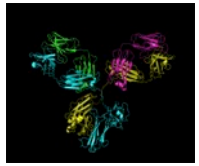
Genome databases

1986: HIV
1994: HPV
1996: Influenza
1998: STD
1999: Chem-Bio

Chromosome 16
Joint Genome Institute

1990's

2000



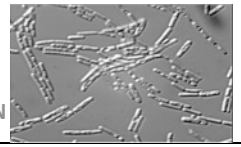
TB Structural Genomics Consortium

BioWatch

2004

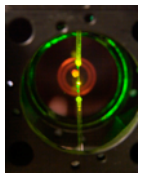
2005

Bioforensics Analysis Research and Development Center

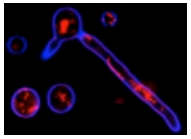


High Throughput Laboratory Network

2006

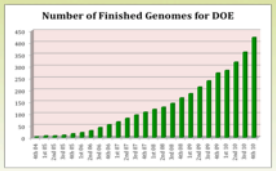


2008
Acoustic Flow Cytometer R&D 100 Award



Genetic sequence of *Trichoderma reesei*

2008

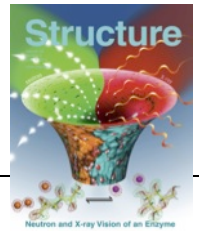


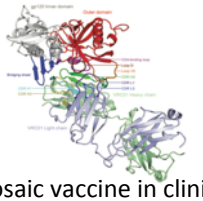
2010
500th Genome Finished

2010
National Alliance for Advanced Biofuels and Bioproducts

NAABB
National Alliance For Advanced Biofuels and Bio-products

2010
Complementary neutron & X-ray crystallography used at LANSCE-PCS

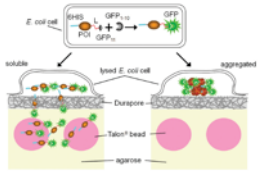




HIV Mosaic vaccine in clinical trials

2011

2011
GFP Reagents



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Infrastructure and Resources

Joint Genome Institute

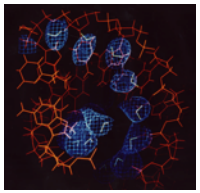
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

DOE User Facility, for world-class genome sequencing and finishing

Center for Integrated Nanotechnologies

DOE User Facility for nanoscale science

Protein Crystallography Station



Neutron crystallography used to determine protein structures (DOE-BES and NIH)

Protein Engineering and Protein Structure/Function Pipelines

Affinity reagent development for selection and screening systems (NIH, DOE)

Theory and Computation

Center for Nonlinear Studies

National Flow Cytometry Resource

>40 Years of innovative flow cytometry development (NIH)

NFCR
National Flow Cytometry Resource

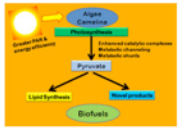
DOE-EERE Consortia

NAABB
National Alliance For Advanced Biofuels and Bio-products

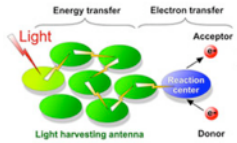
NABC National Advanced Biofuels Consortium
Biofuels for Advancing America

DOE-SC Consortia

Energy Frontier Research Centers



Center for Advanced Biofuel Systems



Photosynthetic Antenna Research Center

New Mexico CONSORTIUM

Los Alamos
NM STATE
NEW MEXICO TECH

National Stable Isotope Resource

Stable isotopes and metabolomics to understand cell regulation and metabolism (NIH)

Bioscience Division

Biosecurity & Public Health Group

- Evaluation of cellular responses to infection
- Advancement of disease characterization
- Biosensor research and development
- Cytometry and spectroscopy-based tools for biomedical applications and fuel production
- Molecular recognition reagents for biomedical, biodefense, public health, and biofuel missions
- Development of widely-distributed techniques for optimizing protein folding, stability, and solubility
- Advancement of protein structure analysis

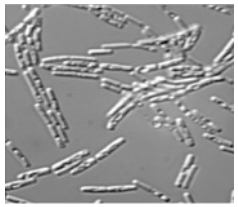
Capabilities

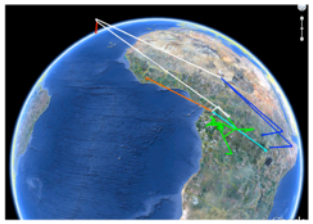
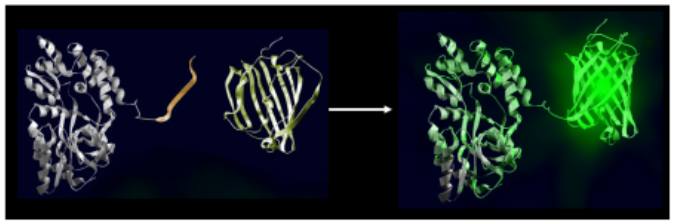
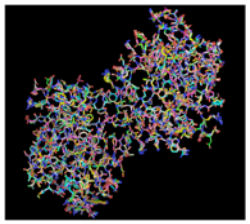
Bioengineering, Protein Design Structure and Evolution

Biosurveillance

Molecular Recognition and Design

Pathogen Science

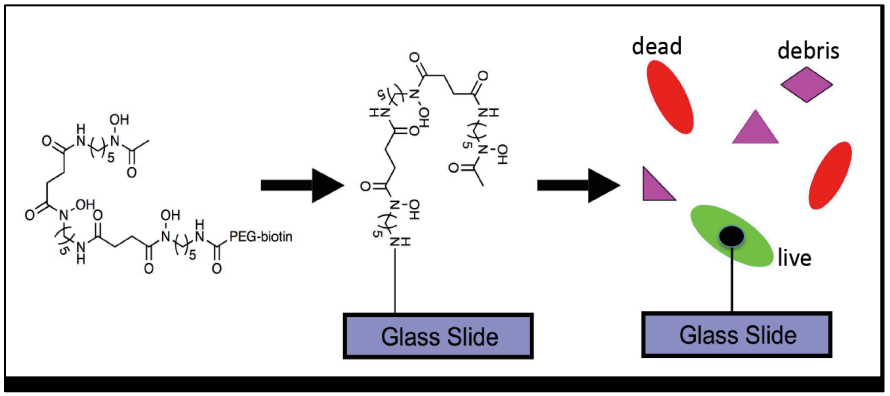



Rapid, fieldable detection of live pathogens

- LANL team used bacterial siderophores to identify bacteria selectively and rapidly in complex matrices *Enabled discrimination between viable bacteria and dead counterparts*
- Team showed that self-assembled monolayers (developed at LANL) could be used to minimize non-specific interactions (i.e. with culture filtrate, serum, and urine)
- Rapid and selective interrogation of bacterial viability is critical to many fields
  - Food safety
  - Rapid determination of the efficiency of a decontamination process,
  - Efficacy assessment after initial medical intervention to infection
  - Detection of exposure to a biological threat agent.

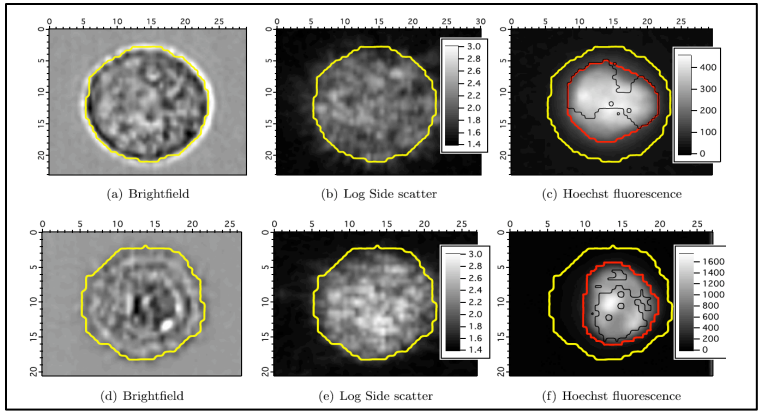
"Determination of Bacterial Viability by Selective Capture using Surface-bound Siderophores," ***Advances in Biological Chemistry*** *2*, 396 (2012); doi:10.4236/abc.2012.24049



Schematic representation of the proposed approach. The siderophores (desferrioxamine B) are tethered to functionalized glass slides and then exposed to a mixture of viable and dead *E. coli*. Only living *E. coli* bind to the siderophores, enabling determination of bacterial viability.

Detecting precancerous and cancerous lesions

- Applications of acetic acid/acetowhitening used to:
  - Detect precancerous cervical lesions
  - Study cancerous growths
- LANL team measured changes in light scattering specific to the nucleus and the cytoplasm of two cancerous cell lines to better understand 'acetowhitening' mechanism
- Results should lead to a better understanding of acetowhitening and potentially the development of adjunct techniques to improve the utility of acetic acid application
- On-going project to develop non-intrusive optical spectroscopy as real-time diagnostic for cervical dysplasia
  - Current trial at Albert Einstein School of Medicine



(a) to (c) Images of a SiHa cell not exposed to acetic acid. (d) to (f) Images of a SiHa cell exposed to 0.6% acetic acid. The scales on the x and y axes are in microns. Side scatter intensity is presented on the same log scale for (b) and (e).

"Effects of Acetic Acid on Light Scattering from Cells," ***Journal of Biomedical Optics*** 17, 085002 (August 2012).

Advances in detection of Tuberculosis

- As incidence Tuberculosis and drug-resistant TB climb, better detection and diagnosis are critical
- Conventional immunoassays cannot distinguish between active and latent infection (thus can also detect people who have been immunized)
- LANL team developed novel "sandwich" immunoassay for three pathogen-specific biomarkers of tuberculosis
  - Allows for further discrimination of active from latent disease or exposure
- Using waveguide-based platform developed at LANL, the team enhanced sensitivity of detection as required for the measurement of the small concentrations of pathogen biomarkers in patient urine

"Rapid detection of Mycobacterium tuberculosis biomarkers in a sandwich immunoassay format using a waveguide-based optical biosensor." ***Tuberculosis*** 92 (2012) 407-416. doi:10.1016/j.tube.2012.05.009

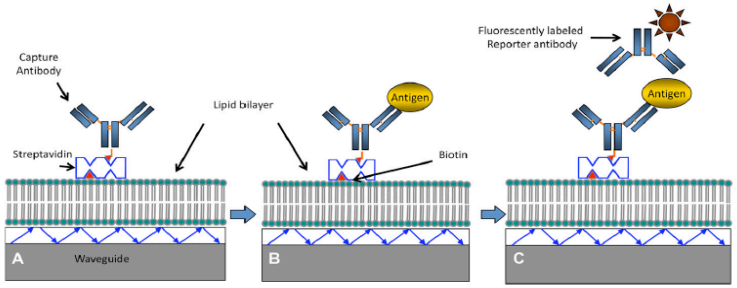
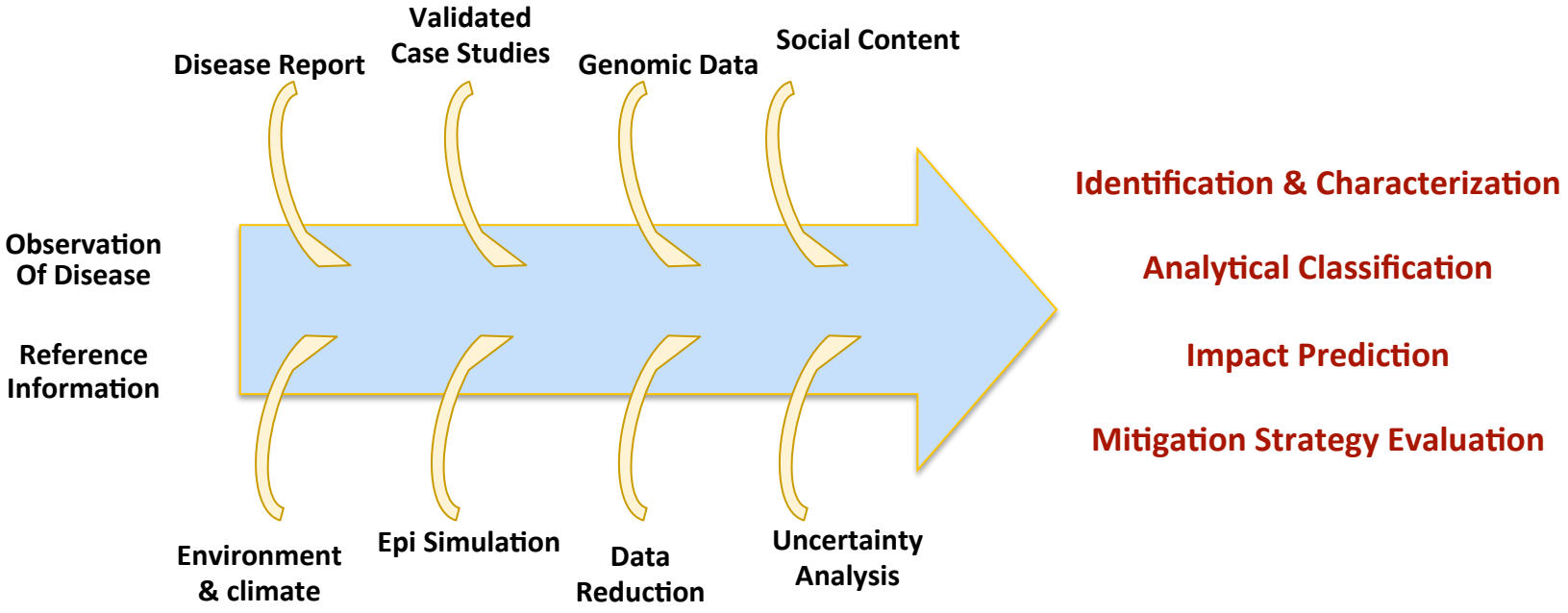


Illustration of the sandwich immunoassay on a waveguide. A) Waveguides are prepared by binding of capture antibody using biotineavidin chemistry. B) Addition of the sample containing the antigen results in specific binding. C) Fluorescently-labeled reporter antibody used to indicate the presence of the TB biomarker.

Community Information Workbench for Global Biosurveillance

- To achieve global biosurveillance of disease, integration of existing data, and science and technology into a common plaform and interface is essential
- Successful proof-of-concept achieved through *BioPASS* project (US Dept of State)
- Highly accomplished, interdisciplinary team (internal and external to LANL) contributes expertise in all areas of biosurveillance



Bioscience Division

Bioenergy & Biome Sciences Group

- Advancement of viable algal fuel production
- Investigation of cellulose structure and cellulase function for biomass degradation
- Biochemical conversion for commodities and fuels
- Climate science through microbial ecology
- Advancement of genome sequencing and finishing, single cell genomics, and whole genome amplification
- Assembly and analysis of metagenomic data

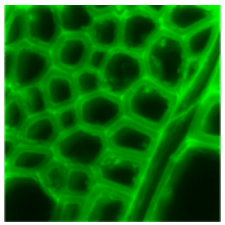
Capabilities

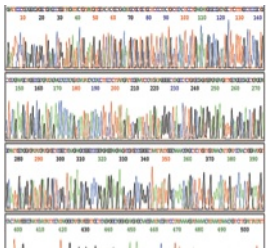
Bioinformatics and Analytics

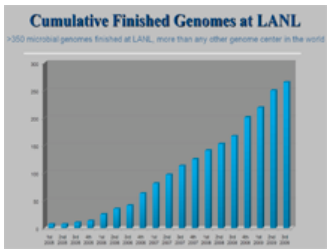
Biomass and Biodiversity

Biophysical Chemistry

Genome Technologies







Bioscience Division

Key Laboratory Capabilities

Leveraging National Assets
to Advance the Production of Biofuels from Algae

- Scientific Expertise
- Interdisciplinary Collaborations
- Unique Facilities
- High-end Instrumentation

LANSCE
Los Alamos Neutron Science Center

- 3 DOE User Facilities
- 4 Relevant NIH Centers
- 4 National DOE Consortia
- 20+ LDRD Investments
- New Mexico Consortium

NATIONAL INSTITUTES OF HEALTH

National Institute of General Medical Sciences
Biomedical Technology Research Centers

NAABB
National Alliance For Advanced Biofuels and Bio-products

LDRD
innovation for our nation

New Mexico CONSORTIUM

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Los Alamos NATIONAL LABORATORY EST. 1943 NNSA

Bioscience Division

LANL Investments in BioEnergy R&D

- *$32M+ in LDRD*
- *Strategic Hire*
- *New research facility*

Algal Biology Program at Los Alamos gets a star
Richard Sayre joins LANL for plant research

LOS ALAMOS, New Mexico,

October 11, 2011—Richard Sayre, one of the nation's top specialists in algae and energy-producing plant research, has joined the Bioscience Division of Los Alamos National Laboratory to help boost cutting-edge research in this area. Cited by Nature magazine as "one of five crop researchers who could change the world," Sayre brings a crew of postdoctoral researchers and a range of funding to LANL.

New Mexico Consortium breaks ground on biology lab

May 31, 2012—U.S. Senator Tom Udall (D-NM) recently spoke at the groundbreaking ceremony marking the start of construction on the New Mexico Consortium's biological research facility at Entrada Park.

New Mexico Consortium BioLab

- Construction is underway for the New Mexico Consortium BioLab at Entrada Park in Los Alamos
- Collaborative effort between NMC, LANL, Los Alamos County, and Los Alamos National Bank
- 27,000 square foot laboratory and office facility including a 4,000 square foot research greenhouse
- Key facility for algae and plant research at NMC and LANL

BioLab groundbreaking in May 2012

Bioscience Division

INCITOR
MAKING RENEWABLE FUELS A REALITY

life technologies™

Collaborations and CRADAs

MESA TECH INTERNATIONAL INC

- LANL: strong track-record from basic, interdisciplinary research to systems integration
- Knowledge and experience to effectively partner both internally and externally
- Together we deliver integrated, innovative solutions for global energy alternatives
- Types of collaboration:
  - Memoranda of understanding
  - CRADA
  - Work for others

SOLIX
Fueling a better world

American Qualex
Antibodies
Molecular

Chevron
Human Energy™

Akonni Biosystems

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

P&G

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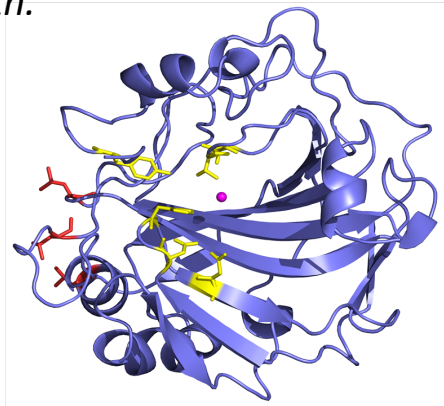
Thermostabilized enzyme created for biofuels production

- Carbonic anhydrases (CAs) are ubiquitous enzymes that regulate the interconversion of carbon dioxide (CO_2) and bicarbonate
- Identified specific amino acids on the outer surface of HCA II that, when mutated, give enhanced physical properties to this enzyme
- LANL Scientists created a variant of HCA II in which they substituted a cluster consisting of three hydrophobic (repelling water) amino acid residues with more polar/charged ones
- This combination of designed mutations creates HCA II variants with improved thermal stability and enzymatic activity—*creating potential for use in biofuel production where CO_2 capture is needed for feedstock growth.*

"Kinetic and structural characterization of thermostabilized mutants of human carbonic anhydrase II" ***Protein Engineering: Design & Selection*** June 12, 2012, DOI: 10.1093



Ribbon diagram of HCA II in blue. The 3 surface Leu residues that were mutated to confer thermal stability are shown in red ball-and-stick. The active site Zn and residues are shown in magenta and yellow, respectively. Image was shown on the Journal cover.

Making gas from biomass

- Efficient conversion of non-food biomass into transportation fuels and chemical feedstocks can potentially
  - Reduce our dependence on foreign oil
  - Ensure the long term availability of renewable starting materials for consumer products
  - Reduce the production of greenhouse gasses
- One aspect of non-food biomass transformation is the opening of furan rings—ubiquitous molecules found in cellulose/carbohydrate derived fuel precursors.
  - Difficult because it requires high temperatures and pressures
- Los Alamos team investigated an alternative approach to opening the furan rings using a simple catalyst, hydrochloric acid, in low concentrations (10 molar %). *Potential for better catalysts and processes for biomass fuels and commodity chemicals on large scales*

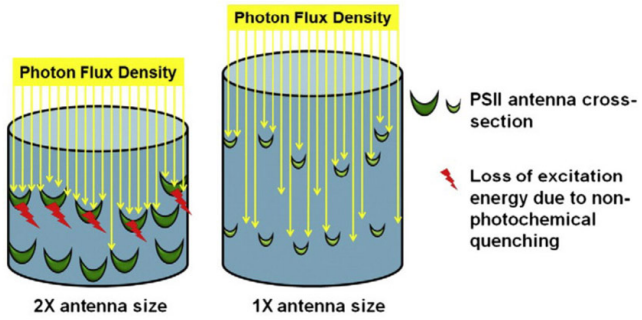
"Functional group dependence of the acid catalyzed ring opening of biomass derived furan rings: an experimental and theoretical study." ***Catalysis Science & Technology,*** DOI: 10.1039/c2cy20395b.

Artist's conception of the process of converting plant biomass to alkanes for fuel as shown on the Journal cover.

Microalgae for photosynthesis

- Challenges remain before microalgae can be a commercially viable alternative to current methods of feedstock and fuel production
- Photosynthetic efficiencies in microalgae cultures are 2-3 times lower than their theoretical potential due to differences in the fast rate of photon capture and the much slower downstream kinetics of photosynthetic electron transfer and carbon fixation
- LANL researchers and colleagues sought to create transgenic microalgae that could optimize photosynthetic light energy utilization
- LANL team also calling for assessments to address potential risks of the use of GM algae, such as those with reduced antennae sizes

Optimization of photosynthetic light energy utilization by microalgae, (2012) ***Algal Research***: http://dx.doi.org/10.1016/j.algal.2012.07.002



A model for light absorption and utilization by algae with large and truncated antennae at saturating light intensities shows a loss of excitation energy below the surface in algal cultures with larger antennae.

Response of soil biological crust to temperature and precipitation

- In plant-sparse ecosystems, complex communities of cyanobacteria and other bacteria, algae, fungi, lichens, and mosses form biological crusts (biocrusts) on the soil surface
- Biocrusts play an essential role in soil stability and albedo, water infiltration, seed germination, and carbon and nitrogen inputs for soil enrichment
- LANL researchers used *nifH* gene-based surveys to examine variation in the diazotrophic (nitrogen-fixing organisms) community of biocrusts in response to season
- Showed that loss or reduction of biocrust cover could have major negative effects on the nutritional status of the carbon- and nitrogen-limited dryland soil—thus changing feedbacks to the climate system.

"Response of Biological Soil Crust Diazotrophs…" ***Frontiers in Microbiology*** *3,* October 2012, Article 358; doi: 10.3389/fmicb.2012.00358 **AND** "Increased Temperature and Altered Summer Precipitation…" ***Global Change Biology*** *18,* 2583 (2012); doi: 10.1111/j.1365- 2486.2012.02709.x

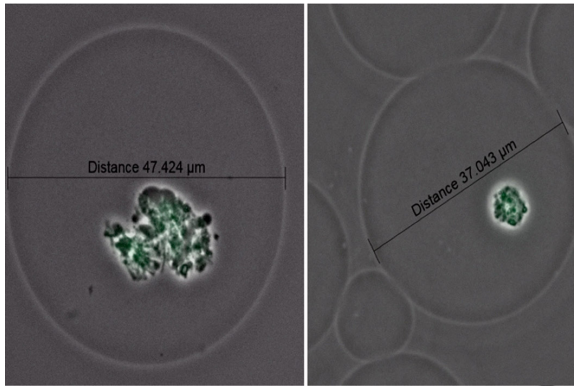
Healthy biocrust in a control plot

Soil surface in altered precipitation plot after 5 years

New culturing tool reveals a full genome from single cells

- Most types of bacteria cannot grow in the laboratory as a pure, isolated culture due to complicated interactions that support their growth—this making sequencing difficult
- LANL team demonstrated a novel approach for fully sequencing genomes of microorganisms found in complex communities
- Using gel microdroplets (GMD), the team created dozens to hundreds of identical cells from single cells, while keeping such cells separated from the rest of community and maintaining the cells' ability to communicate with other community members
  - The characteristic pores and channels of the agarose-based GMD allow for the movement of nutrients, chemical signals and metabolic wastes to and from the living cell as if it were in its natural environment.

"Nearly finished genomes produced using gel microdroplet culturing reveal substantial intraspecies genomic diversity within the human microbiome." 14 March 2013, ***Genome Research,*** doi/10.1101/gr.142208.112

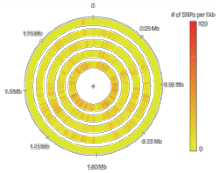


Two GMD containing gut-community microcolonies are shown, with green fluorescence marking the DNA

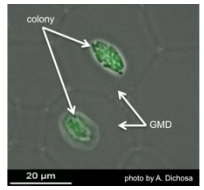
Lab-wide Bioscience Capabilities

SEQUENCING AND GENOMICS

Uncovering biological novelty via sequencing and comparative genomics



- Leading the field in generating libraries from low input DNA (including single cells)
- Proven ability to generate high quality data, and assemblies from difficult sources (such as RNA viruses or algae and insects)
- Detailed data analysis from all sample types, including assembly, annotation, and data representation (secure interactive website)
- Able to compare large datasets and correlate results with metadata



Highlights

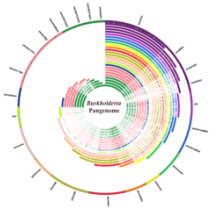
- >150 publications (since 2011)
- 594 microbial genomes sequenced to date
- 4 algal genomes drafted and improved, 5 more in progress
- >250 transcriptome projects completed (bacterial, algal, human, and metatranscriptomes
- Other eukaryotic genomes/exomes (e.g. human cancer, toxicology, host-pathogen response)
- 100's of single cell projects completed (last 12 months)
- >50 metagenomes drafted and >200 analyzed (last 12 months)

Why LANL?

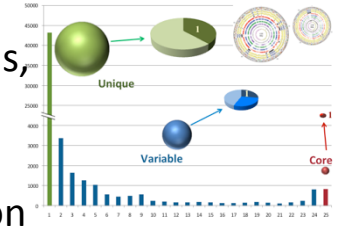
From sample preparation to sequence to analysis, LANL has excelled as a single-source provider of (meta)genomic/transcriptomic information and analysis

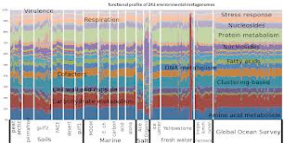
BIOINFORMATICS

Sequence analysis using advanced algorithms, software and computational hardware



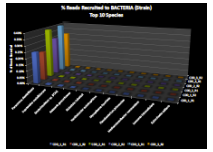
- World-class databases and tools for HIV, hepatitis C virus, hemorragic fever viruses, and Influenza sequences
- Leader in metagenome data analysis (assembly, kmer-based signature calculations, binning, classification, comparisons)
- Pioneers in genome improvement for microbes and single cells
- Detailed bacterial genome assembly, annotation, analysis, and data representation on a secure interactive website

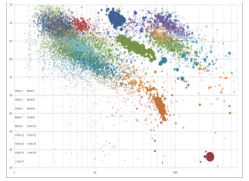




Highlights

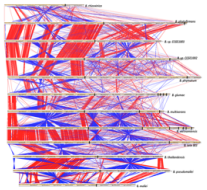
- Novel algorithm recently claimed prestigious R&D100 award
- >60 publications (last 2 years)
- Developed workflows now used in production at major sequencing centers (e.g. JGI) to perform metagenome assembly for hundreds of projects
- Pipelines developed to rapidly process metagenomic reads for taxonomic and functional classification
- Developed methods to automatically improve and annotate draft or complete isolate genomes, single cell genomes, as well as metagenomes





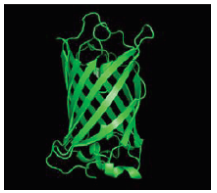
Why LANL?

LANL has a rich bioinformatic history (e.g. Genbank) and has demonstrated a full range computational expertise tailored to customer needs

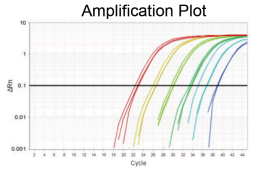


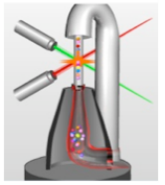
ASSAY DEVELOPMENT

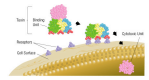
Assays for Diagnostics and Bioforensics: Application to Biosurveillance

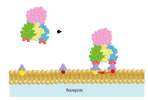


- ***Signature and assay design pipeline:*** LANL ProSig and ThermonucleotideBLAST algorithms allows for design of genetic-based detection assays for biodetection and bioforensics.
- ***Next Gen DNA sequencing and analysis pipeline:*** for rapid sequence determination and analysis of biothreat/ public health pathogens.
- ***Molecular recognition reagents****:* Phage and yeast display to select hundreds of human antibodies or peptides against specific targets.
- ***Novel biosensor and detection platforms:*** Wave guide sensors; fluorescent nanoclusters; custom nanofluidics.



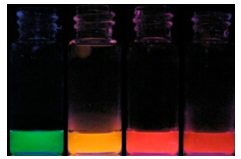






Highlights

- Over 400 peer-reviewed articles in the area of pathogen detection , Institutional H index of 38 (2007-2012)
- 153 pathogen genome sequences
- Fluorescent NanoCluster Beacons: winner of 2011 R&D100 award; fieldable, inexpensive, and sensitive.
- Split-GFP assays developed to characterize protein-protein interactions
- Application to real-world clinical samples: tuberculosis, HIV, Influenza



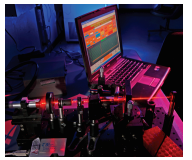
Why LANL?

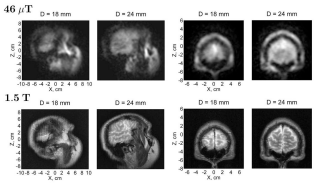
LANL has the full range of capabilities to support assay development for biodetection, biosurveillance and bioforensics.

SENSOR & DETECTION PLATFORMS

Translational Research for Pathogen Detection and Identification

- Flow cytometry pioneered at Los Alamos. The NIH supported National Flow Resource supported the development of FCM
- Magnetoencephalography based on SQUID detectors pioneered at LANL
- Brain imaging using ultra-low field magnetic resonance imaging first demonstrated at LANL; super-paramagnetic resonance and MRI unique to LANL
- Field-ready sensors based on optical waveguides developed and demonstrated for pathogen detection and diagnosis (Swanson & Grace)
- Manipulation of biological particles using acoustic fields demonstrated for cell separation and storage, harvesting of microalgae and cell disruption





Highlights

- ~250 publications in flow cytometry with ~ 6500 total cites and an institutional h-index of 43
- 25 issued patents in flow cytometry, magnetoencephalography, magnetic resonance imaging, and optical biosensors combined.
- Licensing of intellectual property has led to five start-up companies.
- Ultra-low-field NMR imaging has resulted in 12publicationsand two patents and a spin-off company for Homeland Security applications.

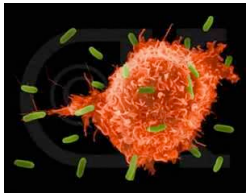
Why LANL?

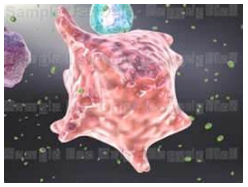
LANL has a long history of translational research for the development of sensors and detection platforms for biomolecules

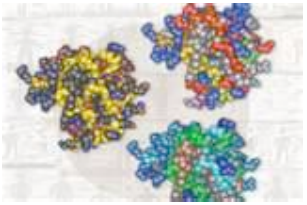
HOST PATHOGEN SCIENCE

Fundamental discovery and applications in host-pathogen science

- Mosaic protein strategy to develop vaccines against viral pathogens, including HIV, hepatitis C, and Ebola
- Mathematical biology of viral dynamics and evolution
- Multi-scale modeling of host-pathogen interactions, host immunity, and within-host infection
- RNA interference (RNAi) screens to identify key human genes that function in pathogenesis and host response
- Characterization of pathogen and host small RNAs as potential targets for therapeutic development

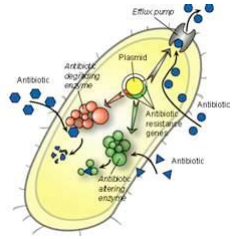






Highlights

- >520 peer-reviewed articles in host-pathogen science, institutional h-index >96
- Phase I clinical trials of mosaic protein vaccine against HIV, in collaboration with Duke University
- High-Throughput Lab Network w/UCLA for influenza surveillance – design and construction of robotic systems for pathogen handling
- Novel strategies to design or identify anti-microbial proteins, small molecules, and metabolites to inhibit pathogen growth and host infection

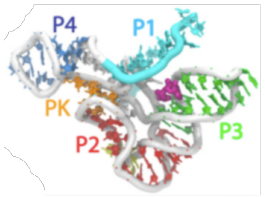


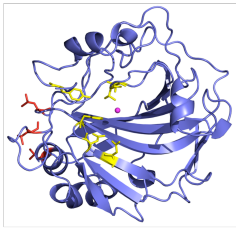
Why LANL?

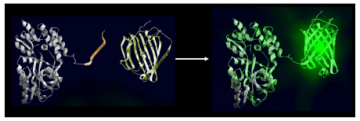
LANL has a full range of capabilities in experimental and theoretical biology to study mechanisms of infectious disease

STRUCTURAL BIOLOGY

Determining the three-dimensional macromolecular structure of biomolecules leads to the understanding of their function



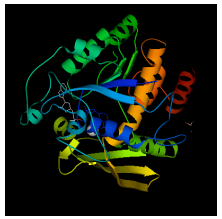




- Structural genomics' complete 'gene to structure' pipeline enables accelerated protein structure analysis
- Neutron crystallography at the Protein Crystallography Station allows determination of the position of hydrogen atoms—key to the mechanism of enzymes & ribozymes.
- Stable isotope capability enables NMR structure determination of larger proteins.
- Large-scale simulations using enhanced sampling methods on high-performance computing platforms produce new insight in the dynamics and mechanism of biomolecular complexes.

Highlights

- >350 peer-reviewed articles in structural biology since 1997. Institutional h-index 53, cites ~ 17,000
- 29 *de novo* protein structures & 26 protein-ligand complex structures made in the last 5 years
- Protein Crystallography Station has 28 published neutron structures, more than half the total produced world wide.
- Geoff Waldo/GFP-based methods for protein engineering featured at Nobel Symposium 2008



Why LANL?

Using this complete collection of tools, LANL is in a unique position to create a comprehensive understanding of macromolecular structure and function